ACTA UNIVERSITATIS LUNDENSIS
SECTIO II 1966 No. 7
MEDICA, MATHEMATICA, SCIENTIAE RERUM NATURALIUM

NERVOUS AND HORMONAL CONTROL OF SALIVARY GLANDS IN RATS

BY

PER OHLIN

LUND 1966
C. W. K. GLEERUP, SWEDEN

Acta Universitatis Lundensis, Sectio II
Medica, Mathematica, Scientiae rerum naturalium
edited by
the Royal Physiographic Society of Lund
will be published from 1964 as a continuation of
Lunds Universitets Årsskrift, N.F. Avd. 2
and will contain papers in
medicine, mathematics and the natural sciences

The numbers, each consisting of a single
paper, are issued at irregular intervals.

ACTA UNIVERSITATIS LUNDENSIS
SECTIO II 1966 No. 7
MEDICA, MATHEMATICA, SCIENTIAE RERUM NATURALIUM

NERVOUS AND HORMONAL CONTROL OF SALIVARY GLANDS IN RATS

BY

PER OHLIN

FROM THE INSTITUTE OF PHYSIOLOGY,
UNIVERSITY OF LUND, SWEDEN

LUND 1966

C.W.K. GLEERUP, SWEDEN

Read before the Royal Physiographic Society, January 18, 1966

UNIVERSITEITSBIBLIOTHEEK - LEIDEN -

LUND 1966
HÅKAN OHLSSONS BOKTRYCKERI

This thesis is based upon the following papers:

1. Secretory responses of innervated and denervated submaxillary glands of rats. Acta Univ. Lund. II. No. 23 (1965).
2. Salivary secretion of the major sublingual gland of rats. Experientia *21:* 408–409 (1965). (Together with PEREC, C.).
3. Isoprenaline as a secretory agent in salivary glands. Acta Univ. Lund. II. No. 17 (1964).
4. The effect of denervation on respiratory enzymes in the submaxillary gland of the rat. Experientia *21:* 447–448 (1965).
5. The effect of treatment with pilocarpine on the weight of innervated and denervated submaxillary glands of rats. Acta Univ. Lund. II. No. 6 (1966).
6. Effects of treatment with isoprenaline or pilocarpine on the secretory responses of the parasympathetically denervated submaxillary gland of the rat. Published in J. oral Therap. Pharmacol. 2 (1966).
7. Effects of isoprenaline treatment on secretory responses and respiratory enzymes of the submaxillary gland of the rat. Published in J. oral Therap. Pharmacol. 2 (1966).
8. The effect of hypophysectomy on respiratory enzymes in the submaxillary gland of the rat. Quart. J. exp. Physiol. *47:* 238–243 (1962).
9. Effect of hypophysectomy or treatment with thyroxine or testosterone on secretory responses and respiratory enzymes of the submaxillary gland of the rat. Quart. J. exp. Physiol. *50:* 446–455 (1965).

These papers will be quoted in the text as (1), (2), etc.

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41545309_0003

Glandular cells may be activated by nerve impulses or by chemical agents in the blood supplying the gland. Some gland cells may in addition be endowed with an inherent ability to secrete even in the absence of external stimuli, "spontaneous secretion", according to Babkin (1950).

In salivary glands secretion is normally evoked by autonomic nerves. Parasympathetic fibres play the main role, but some salivary glands, such as the cat's submaxillary gland (Langley, 1878–79) and the rabbit's parotid gland (Nordenfelt and Ohlin, 1957) are supplied with sympathetic secretory fibres as well. Certain glands possess the ability to secrete spontaneously, e. g. the parotid gland of sheep (Eckhard, 1867), the cat's sublingual gland (Emmelin, 1953) and the rabbit's submaxillary gland (Nordenfelt and Ohlin, 1957), but in most salivary glands studied no secretion occurs in the absence of external stimuli. Secretion of saliva can also be elicited by administration of certain substances. As a rule these belong to the group of parasympathomimetic agents, for instance acetylcholine, methacholine or pilocarpine; salivary glands with sympathetic secretory fibres also respond to sympathomimetic agents, such as adrenaline and noradrenaline. These two agents are the only hormones present in the blood known to cause a flow of saliva, but under ordinary conditions their concentration in the blood will be subthreshold as far as secretion of saliva is concerned.

In recent years increasing attention has been paid to the fact that efferent nerve fibres, in addition to their immediate effect, exert some long-term action on the effector cells. From observations on chronically denervated salivary glands it is obvious that the nerves affect the size and the structure of the glands; regulate the responsiveness of the cells to secretory stimuli; influence the secretory capacity and the activity of various enzyme systems (see Burgen and Emmelin, 1961). Similarly, it has been shown that several hormones are of importance for the size and structure and the histochemistry of salivary glands (see Shafer and Muhler, 1960).

Morphological changes, brought about by denervation or by extirpation of certain endocrine glands or treatment with hormones, have been studied mainly in rats and mice. Very little is known about functional changes in the glands of these species, the reason being that it is difficult to study salivary secretion from separate glands in these small animals. Our knowledge of the physiology of the salivary glands, on the other hand, is to a great extent based on obser-

vations on dogs and cats. It would seem desirable to correlate the various structural and functional changes caused by interference with the nervous or hormonal control in glands of the same species.

The present investigation starts with a series of experiments showing that it is possible to cannulate the ducts of the submaxillary (1, 3) and sublingual glands (2) in anaesthetized rats and therefore to study the secretion. The sublingual gland was found to secrete spontaneously. This was not the case with the submaxillary gland, which therefore appears more suitable for studies of the effect of external influences on the gland cells and has been used in most of the experiments in the present investigation. The submaxillary gland was found to be richly supplied with sympathetic secretory fibres. It is therefore necessary to take both parasympathetic and sympathetic nerve fibres into account. Secretion was found to occur not only after parasympathomimetic drugs and adrenaline and noradrenaline, but also after the β-receptor stimulating agent isoprenaline, as observed previously by SCHNEYER (1962).

In the subsequent investigation the effects of the following procedures were studied:

1. Parasympathetic denervation
2. Treatment with pilocarpine
3. Sympathetic denervation
4. Treatment with isoprenaline
5. Hypophysectomy
6. Treatment with thyroxine
7. Treatment with testosterone

The effects of these procedures on the weights of the glands and on the secretory responses to drugs were studied. When examining the secretory effects particular attention was paid to the threshold doses of the drugs and to the maximal flow rate of saliva from the glands, using pilocarpine as a stimulating agent. The activities of certain respiratory enzymes, succinic dehydrogenase, cytochrome oxidase and fumarase, were also estimated in a number of experiments. The effects observed were correlated to the histological changes known from the literature under the various conditions.

Salivary secretion studied in normal glands of rats

Secretion of saliva in rats has previously been studied mainly after administration of big doses of sialagogue agents, such as pilocarpine. The saliva has been collected in the oral cavity (Bernarde *et al.*, 1956; Shafer *et al.*, 1958; Holloway and Williams, 1963) or from the cut secretory duct (Schneyer and Schneyer, 1959). In the present investigation the secretory ducts of the rat's submaxillary (1, 3) and sublingual glands (2) were cannulated using fine glass cannulae which permits an accurate estimation of the secretory responses of these glands. A similar technique has recently been used by others on the submaxillary gland (Drum, 1963; Junqueira, Toledo and Saad, 1964).

A continuous secretion of saliva was obtained from the sublingual (2) but not from the submaxillary gland (1). This continuous secretion was found to be an example of spontaneous secretion. The evoked secretory responses were superimposed on this spontaneous secretion (2).

Stimulation of the parasympathetic secretory nerve of the rat's submaxillary gland evoked a lively flow of saliva which was maintained even when the stimulation was continued for several minutes. Administration of parasympathomimetic agents, such as acetylcholine, methacholine and pilocarpine, also elicited secretory responses from the gland (1). Similarly, parasympathomimetic substances evoked secretion of saliva from the sublingual gland (2); it was considerably smaller than that from the submaxillary gland, very likely because of the small size of the sublingual gland.

The sympathetic secretory innervation of salivary glands has been a matter of dispute. In some types of salivary glands the flow of saliva caused by sympathetic stimulation is mainly or wholly due to expulsion of saliva from the ducts (Babkin, 1950). In rats sympathetic stimulation or injection of sympathomimetic agents, such as adrenaline and noradrenaline, was found to evoke marked secretory responses from the submaxillary gland. Since the secretion was pronounced and of the same magnitude even when the gland was stimulated repeatedly at intervals (1), it seems unlikely that the responses can be due to mechanical extrusion of saliva. Instead, it may be assumed that the rat's submaxillary gland is supplied with sympathetic secretory fibres. This is supported by the fact that adrenergic nerve fibres have been observed histologically in close contact with the acinar cells of the gland (Norberg and Hamberger, 1964; Norberg and Olson, 1965). In the sublingual gland, on the other hand, sympathomimetic substances did not usually elicit any secretory responses (2). This finding indicates that the sublingual gland lacks a sympathetic secretory innervation, which is in accordance with histological observations (Norberg and Hamberger, 1964).

The secretory responses of the cat's submaxillary gland to nerve stimulation (Emmelin, 1955 a) and the electrophysiology of the gland (Langenskiöld,

1941; LUNDBERG, 1955) indicate a double innervation, i. e. both sympathetic and parasympathetic, of at least some of the effector cells. In rats stimulation of the parasympathetic nerve fibres of the submaxillary gland was found to evoke a more pronounced secretion of saliva than sympathetic stimulation. The flow rate on maximal chorda stimulation did not increase when sympathetic stimulation was added (1). This finding suggests that the sympathetically innervated gland cells can be maximally activated by the parasympathetic nerve fibres. The cells thus seem to have a double innervation. Morphological observations led GLIMSTEDT and HILLARP (1942) to a similar conclusion.

The sympathetic secretory innervation and the secretory responses of salivary glands to sympathomimetic agents have been studied particularly in the cat's submaxillary gland (see BURGEN and EMMELIN, 1961). When examining the rat's submaxillary gland in these respects, an interesting difference between the glands of the two species was found. In cats the secretory response to sympathetic stimulation is abolished by a sympatholytic agent like dihydroergotamine (EMMELIN, 1955 b; MARTIN, 1964); in rats, however, it was only reduced by α-receptor blocking drugs (1). In contrast to the gland of the cat (EMMELIN and MUREN, 1951; MARTIN, 1964; 3), that of the rat was found to respond markedly not only to adrenaline and noradrenaline, but also to the β-receptor stimulating agent, isoprenaline (1, 3). These findings suggest that the sympathetic secretory nerve fibres of the rat's submaxillary gland act on two types of catechol amine receptors, those of α- and β-type according to AHLQUIST (1948). Since the submaxillary gland of rats is supplied with α- and β-receptors it presents a suitable tool for studies of the pharmacology of sympathomimetic and sympatholytic agents. The gland has been used for this purpose, e. g. to estimate the action of β-blocking agents, such as dichloroisoprenaline (3) and pronethalol (EMMELIN, HOLMBERG and OHLIN, 1965).

Nervous control

1. *Effects of Parasympathetic Denervation*

The parasympathetic nerve supply is known to be important for the structure and function of salivary glands. Thus, when the action of the parasympathetic fibres on the gland cells is abolished by denervation, profound structural changes ensue. In the rat's submaxillary gland the two main structures connected with secretion, the acini and the tubules, show degenerative changes after denervation (Snell, 1960). The weight of the gland is diminished (Houssay *et al.*, 1962; Junqueira, Toledo and Saad, 1964; Wells and Peronace, 1964). The structural modifications of the gland after preganglionic parasympathetic denervation were found to correspond to a decrease in the weight of the gland by about 35 per cent in 3 weeks (1, 4).

The sensitivity of gland cells to secretory agents is dependent on the activity in the nerves (see Emmelin, 1965). According to Cannon's "Law of Denervation" a supersensitivity to stimulating substances develops after denervation. In the rat's submaxillary gland parasympathetic denervation was found to cause a supersensitivity not only to acetylcholine, methacholine and pilocarpine but also towards adrenaline and isoprenaline (1, 3). In those cells which are responsive both to parasympathomimetic and sympathomimetic drugs the parasympathetic nerve fibres obviously influence the sensitivity to both types of stimulating compounds.

The maximal rate of flow of saliva elicited by pilocarpine from the submaxillary gland of rats was not found to be changed by parasympathetic denervation carried out three weeks earlier (1). This was true in spite of a considerable atrophy of the gland. In this respect the submaxillary gland of the rat differs from that of the cat, in which parasympathetic denervation markedly reduces the maximal flow rate in response to pilocarpine (Emmelin, Malm and Strömblad, 1960).

Section of the efferent nerves of salivary glands is known to affect the activity of various enzymes. In the parasympathetically denervated salivary glands of cats and rabbits the activities of some respiratory enzymes, succinic dehydrogenase, cytochrome oxidase and fumarase, have been found to be reduced (Nordenfelt, Ohlin and Strömblad, 1960). The total activities of these enzymes were decreased in the denervated submaxillary gland of rats, as well. The decrease in activities of succinic dehydrogenase and fumarase was more pronounced than the atrophy of the gland and thus the concentrations of these two enzymes were lowered; the activity of cytochrome oxidase was decreased corresponding to the reduction in the weight of the gland (4).

2. *Effects of Treatment with Pilocarpine*

Under physiological conditions the reflexly elicited secretion of salivary glands is mainly due to impulses in the parasympathetic nerve fibres (Babkin, 1950). The secretory activity of the glands can be supposed to be almost completely abolished after parasympathetic denervation. The role played by the decrease of the secretory activity in changing the structure and function of denervated glands may be studied after treatment with a long-acting parasympathomimetic substance, such as pilocarpine, which preserves a secretory activity in the glands. The submaxillary gland of rats was used to study the effect of such a treatment (5, 6).

Previous observations by other workers indicate a close relationship between the weight of salivary glands and the secretory activity. In cats the morphological modifications (Emmelin, Jacobsohn and Muren, 1951) and the weight decrease (Strömblad, 1956 a and b) after parasympathetic denervation are not pronounced if the secretory activity is maintained by treatment with pilocarpine. It has also been found that the weight of the rat's salivary glands is dependent on the consistency of the diet and thus the organ activity; the size of the gland decreases when the animals are on a liquid diet (Hall and Schneyer, 1964). In the present investigation it was shown that pilocarpine treatment of rats for 2 weeks increased the weight of the normally innervated submaxillary gland and prevented the atrophy of the gland otherwise occurring after parasympathetic denervation (5). Bixler, Webster and Muhler (1958) and Chan (1964) who administered pilocarpine repeatedly to rats, found no marked changes in structure or weight of the normally innervated submaxillary glands; these investigators, however, used considerably smaller doses of pilocarpine than those given in the present experiments (5).

The amount of secretory agents acting on the gland cells, normally mainly acetylcholine liberated from the parasympathetic nerve endings, seems to determine the sensitivity of the effector cells (see Emmelin, 1965). Thus, after preganglionic parasympathetic denervation when the liberation of acetylcholine is reduced, a supersensitivity to secretory agents develops. Support of this assumption was obtained from denervated glands of cats (Emmelin and Muren, 1952). As in cats, the supersensitivity of the parasympathetically denervated submaxillary gland of rats was found to be abolished by treatment with pilocarpine (6).

3. *Effects of Sympathetic Denervation*

The sympathetic nerve supply of salivary glands is known to affect the structure of the glands. The effect of sympathetic denervation on the weight of the gland varies in different species. Thus, after sympathetic denervation the weight of salivary glands in cats is increased while it is decreased in

rabbits. The weight of the denervated submaxillary gland of rats was found to be decreased by about 10 per cent after 3 weeks (1) which confirms findings of others (Wells, Handelman and Milgram, 1961; Wells and Voelkel, 1963). The degenerative changes of this gland after sympathetic denervation are confined to the acini (Snell and Garrett, 1958).

The sympathetic as well as the parasympathetic nerve fibres of salivary glands are known to control the sensitivity to secretory agents. In the sympathetically denervated submaxillary gland of rats the sensitivity to sympathomimetic agents, such as noradrenaline (1) and isoprenaline (3), was markedly increased while that to parasympathomimetic substances was less affected (1). Some degree of sensitization even to parasympathomimetic agents could be expected, since supersensitivity following denervation is known to be unspecific. Similar observations have previously been made on cats (Emmelin and Engström, 1960).

The maximal rate of flow of saliva from the rat's submaxillary gland after injection of pilocarpine was found to be diminished (1). This reduction corresponded quantitatively to the loss of weight of the gland. In cats, on the other hand, sympathetic denervation is known to cause a slight increase in the maximal flow rate (Emmelin, Malm and Strömblad, 1960); in this species sympathetic denervation is followed by a small increase in the size of the gland.

In the sympathetically denervated submaxillary gland of the rat the activity of cytochrome oxidase was particularly affected. The decrease was even larger than the corresponding reduction in weight of the gland. The total activities of succinic dehydrogenase and fumarase of the denervated glands seemed lower than those of normal glands, but the difference was not statistically significant (4). These observations agree with histochemical findings showing that elements which are histologically changed by sympathetic denervation, the acinar cells, show a high activity of cytochrome oxidase but a very low activity of succinic dehydrogenase (Padykula, 1952; Schneider and Person, 1960; Handelman and Wells, 1963).

4. *Effects of Treatment with Isoprenaline*

Isoprenaline given repeatedly to rats is known to cause changes of the salivary glands which in some respects are contrary to those observed after sympathetic denervation. Thus, isoprenaline treatment causes a marked enlargement of the submaxillary and parotid glands, histologically based upon an increase in the size and, according to some authors, also in the number of the acinar cells (Selye, Veilleux and Cantin, 1961; Argonz, 1962; Schneyer, 1962; Wells, 1962; Chan, 1964; Potho and Paasonen, 1964; Barka, 1965). The effect of isoprenaline treatment on the weight of the gland is not abolished by other

known factors which affect the weight of salivary glands, e. g. the sympathetic (Wells, 1962) and parasympathetic nerve supply (6) and the hypophysial gland (Argonz, 1962). It seems reasonable to conclude, then, that isoprenaline acts directly on the glands.

Isoprenaline evokes a marked flow of saliva in rats (Selye, Veilleux and Cantin, 1961; Argonz, 1962; Schneyer, 1962; Wells, 1962; Chan, 1964). Schneyer (1962) found that the secretory responses of the submaxillary gland to isoprenaline were pronounced; this was confirmed in the present investigation (3, 6). On the sublingual gland, on the other hand, the secretory effect of isoprenaline was very small (2). This finding is interesting since it is known that isoprenaline treatment does not increase the weight of the sublingual gland (Schneyer, 1962; Chan, 1964; Potho and Paasonen, 1964). Scarcity or complete lack of β-receptors in the sublingual gland seems to explain this observation.

It might be hypothetized that the considerable enlargement of the submaxillary gland is secondary in some way to the prolonged and increased secretory activity evoked by isoprenaline. However, pilocarpine given for the same length of time as isoprenaline did not increase the weight of the gland though it elicited an even more marked flow of saliva than isoprenaline (6). When the period of treatment with pilocarpine was extended, some small increase in the weight of the submaxillary gland occurred (5). For comparison it may be mentioned that isoprenaline injections during one week caused an increase of the size of the gland by about 200 per cent (7) whereas pilocarpine given during two weeks in doses, more active on the secretion than those of isoprenaline, produced an increase of the gland by 17 per cent only (5).

Treatment with secretory agents, e. g. pilocarpine and adrenaline, decreases the sensitivity of salivary glands to secretory stimuli in cats (Emmelin and Muren, 1952; Strömblad, 1956 a). In rats treated with isoprenaline, however, the sensitivity of the normally innervated submaxillary gland was increased particularly to sympathomimetic substances (7). There is no ready explanation of this finding; it may be that at the time of the estimation of the sensitivity some small amount of this long-acting drug isoprenaline is still retained by the gland.

In spite of the remarkable enlargement of the gland after treatment with isoprenaline, the maximal flow rate in response to pilocarpine was not at all increased (7). Nor did the activity of cytochrome oxidase or fumarase, calculated per total gland, increase during the treatment. The activity of succinic dehydrogenase did increase, but not in parallel with the gain in weight (7).

Hormonal control

Since the finding of the sexual dimorphism of the submaxillary gland of mice (Lacassagne, 1940 a) it has been shown that the hormones of the pituitary gland, chiefly via thyroid gland and gonads, affect the morphology and the histochemistry of salivary glands. In the rat's submaxillary gland the granular tubules are under hormonal influences. Thus, after hypophysectomy they atrophy and the weight of the gland decreases (Gabe, 1950; Sreebny, 1953; Shafer and Muhler, 1955; Wells *et al.*, 1959; Argonz and Corral Saleta, 1961; Kronman, 1963). As to histochemical changes after hypophysectomy the activity of amylase (Shafer and Muhler, 1955; Sreebny *et al.*, 1957) and that of a proteolytic enzyme (Sreebny, 1953; Shafer and Muhler, 1955) which is supposed to be located in the granular tubules, have been found to decrease. Similar modifications of the rat's submaxillary gland have been observed after thyroidectomy or castration (Leblond and Grad, 1948; Grad, 1949; Shafer and Muhler, 1953). On the other hand, treatment with thyroxine and testosterone prevents the changes of the rat's submaxillary gland after hypophysectomy (Shafer, Clark and Muhler, 1956; Sreebny *et al.*, 1957) and after thyroidectomy or castration (Leblond and Grad, 1948; Grad and Leblond, 1949; Shafer and Muhler, 1953). Furthermore, treatment of intact rats with thyroid (Shafer and Muhler, 1956) or testicular hormones (Lacassagne, 1940 b; Shafer and Muhler, 1953 and 1956; Argonz and Corral Saleta, 1961) causes an increase in size of the granular tubules.—Some histochemical changes have been found also in the acini of the rat's submaxillary gland after hypophysectomy. This concerns males (Bixler *et al.*, 1957) but not females (Kronman, 1963); all rats used in the present investigation were females.

5. *Effects of Hypophysectomy*

The weight of the rat's submaxillary gland was found to be decreased by about 50 per cent at 4–6 weeks after hypophysectomy; the body weight was also diminished but by about 15 per cent only (8, 9).

In hypophysectomized rats the threshold of the submaxillary gland to acetylcholine was raised in most animals (9). This finding indicates that the sensitivity of salivary glands to stimulating agents is partly dependent on the hormones of the hypophysial gland. As to the decreased sensitivity to acetylcholine it is interesting to note that an increased activity of cholinesterase has been observed in the cat's submaxillary gland after hypophysectomy (Kahlson and Renvall, 1956).

Hypophysial hormones were found to influence the maximal rate of flow of saliva elicited by pilocarpine in the submaxillary gland. After hypophysectomy the maximal flow rate was very much reduced, by 17 per cent more

than the weight of the gland (9). It seems reasonable to assume that the decrease in maximal flow rate is due to the degenerative changes in the granular tubules and that these structures play a major role when secretion of saliva is elicited by parasympathetic agents. This would agree with the finding that the secretory effect of pilocarpine is well maintained when the acinar cells have atrophied, more or less, after ligation of the salivary duct (Schneyer and Schneyer, 1961).

In the atrophic glands of hypophysectomized rats the total activities of succinic dehydrogenase, cytochrome oxidase and fumarase were found to be decreased. The activities of cytochrome oxidase and fumarase were reduced corresponding to the decrease in the weight of the gland. The activity of succinic dehydrogenase, on the other hand, was diminished even more and thus the concentration of this enzyme was decreased (8). This finding agrees with the histochemical observation that the activity of succinic dehydrogenase is high in the granular tubules (Padykula, 1952; Handelman and Wells, 1963), i. e. the structure which is modified after hypophysectomy.

6. *Effects of Treatment with Thyroxine*

Treatment of intact rats with thyroxine for 3 weeks caused a gain in weight of the submaxillary gland by about 15 per cent in spite of the fact that the body weight was increased by 6 per cent less than that of controls. The effect of thyroxine on the weight of the gland was not abolished by parasympathetic denervation (9).

In rats treated with thyroxine the thresholds of the submaxillary gland to parasympathomimetic agents, acetylcholine and methacholine, were lowered while that to adrenaline was unchanged (9). Since the sensitivity to acetylcholine seems to be decreased after hypophysectomy, the results (9) suggest that the hypophysial gland modifies the sensitivity of the gland to parasympathomimetics at least partly via the thyroid gland.

The maximal rate of flow of saliva evoked by pilocarpine was found to increase by about 80 per cent (9). Thus, the increase in flow rate is considerably larger than the gain in weight of the gland. The effect of thyroxine on the rate of flow seems to be partly dependent on the integrity of the parasympathetic nerves since the maximal flow rate of denervated glands did not increase more than the weight of the gland during treatment with thyroxine (9).

The total activities of respiratory enzymes, succinic dehydrogenase, cytochrome oxidase and fumarase, were slightly increased just as the weight of the gland. The variations in the activity of the enzymes in different glands were fairly large, however, and the increase was not statistically significant (9).

7. *Effects of Treatment with Testosterone*

The rat's submaxillary gland was found to increase in weight by about 30 per cent after treatment with testosterone for 3 weeks; the body weight was also increased but only by 5 per cent more than that of controls. Parasympathetic denervation did not prevent the effect of testosterone on the weight of the gland (9).

Treatment with testosterone was found to lower the thresholds to acetylcholine and methacholine but not that to adrenaline (9), just as did treatment with thyroxine. Thus, the influence of the hypophysis on the sensitivity of the submaxillary gland to parasympathomimetics seems to be exerted via thyroids as well as gonads.

The maximal flow rate in testosterone treated animals after pilocarpine injection increased (9), contrary to what is seen after hypophysectomy and similar to what is observed after treatment with thyroxine. The effect of testosterone differed in one respect from that of thyroxine, however: the maximal rate of flow of saliva in rats treated with testosterone did not increase more than the weight of the gland (9). This effect of testosterone treatment on the maximal flow rate was not abolished by parasympathetic denervation.

The activities of succinic dehydrogenase, cytochrome oxidase and fumarase seemed slightly increased (although not significantly) after treatment with testosterone. As with thyroxine, the increase roughly corresponded to the gain in weight of the gland (9).

Discussion

The present investigation has confirmed and extended earlier work showing that the *structure of the submaxillary gland of the rat* is dependent both on nervous and hormonal factors. A reduction of the weight of the gland can be produced by section of the parasympathetic or sympathetic nerves, or by hypophysectomy. Conversely, an increase in size of the gland is obtained after treatment with the parasympathomimetic drug pilocarpine, the sympathomimetic drug isoprenaline or the hormones thyroxine and testosterone.

It has been suggested that the weight of the gland is dependent on the degree of secretory activity (Hall and Schneyer, 1964). To some extent the present experiments support this view; section of the main secretory fibres, the parasympathetic ones, is followed by a substantial loss of weight and, on the other hand, treatment with the secretory drug pilocarpine causes some increase in the size of the gland. It is obvious, however, that other factors than secretory activity in the gland cells have to be taken into account also, where the regulation of the weight of the gland is concerned. Treatment with isoprenaline causes a very large increase of the gland; it is true that this drug evokes secretion, but when the effects of pilocarpine and isoprenaline were compared it was found that under experimental conditions in which pilocarpine causes more secretion than isoprenaline, the effect of isoprenaline on the size of the gland was much more pronounced than that of pilocarpine. Furthermore, treatment with thyroxine or testosterone, which have no secretory effect, gives rise to hypertrophy of the gland.

As to the *function of the gland*, on which interest was centred in the present investigation, the significance of the autonomic nerves is well-known. Not only do the nerves, and particularly the parasympathetic fibres, cause the secretory responses, they also affect the level of sensitivity of the gland cells. The present experiments reveal, however, that certain hormones play an important role as regards the function of the gland. Some hormones, lacking secretory activity, obviously influence the sensitivity of the gland to secretory stimuli; the level of sensitivity of the gland cells can be changed by hypophysectomy or by treatment with thyroxine or testosterone. In some respects the function of the gland seems to be even more dependent on hormones than on nerves, as illustrated by the observations made when the maximal capacity of the gland to secrete in response to pilocarpine was studied. The maximal rate of flow of saliva was not decreased when the gland had lost 35 per cent of its weight after parasympathetic denervation; it was only slightly reduced by sympathetic denervation; it was not at all increased in a gland enormously enlarged by treatment with isoprenaline. On the other hand, the effect of hormones on the maximal secretory capacity was striking. Hypophysectomy caused an atrophy, reducing the weight of the gland by about

50 per cent; but the maximal rate of flow obtained after injection of pilocarpine was lowered even more. The hypophysial control seemed to some extent mediated by gonadal hormones, as testosterone treatment increased the secretory capacity to the same degree as the weight of the gland. Thyroxine, however, seemed to be more important in this respect; it was found that after treatment with this hormone the maximal flow rate was increased by about 80 per cent, the weight of the gland by about 15 per cent only.

In an attempt to study another functional aspect, the effect of the various operations and treatments on the activity of certain enzymes in the gland was also investigated. It seems reasonable to assume that enzymes involved in energy metabolism are important for the secretory activity and it might be particularly interesting to study their activity in relation to the maximal capacity of the gland to secrete. Hypophysectomy, which markedly reduces the maximal flow rate obtained on stimulation of the gland with pilocarpine, was found to cause a pronounced decrease of the activities of succinic dehydrogenase, cytochrome oxidase and fumarase. However, substantial reductions of the activities of these enzymes were observed after parasympathetic denervation also; nevertheless there was no decrease of the maximal level at which the gland could secrete in response to pilocarpine. A possible explanation of this difference in behavior of the gland after hypophysectomy and after denervation may be found in the fact that hypophysectomy decreases the activity of a great number of enzymes, as shown in liver and skeletal muscle (see Jacobsohn, Larsson and Norgren, 1965). Parasympathetic denervation, on the other hand, does not seem to have this general effect; it has been shown, for instance, that it does not change the glycolytic activity in the submaxillary gland of the rabbit (Strömblad, 1961). Although the present experiments have demonstrated that the activity of some respiratory enzymes is dependent both on nervous and hormonal factors acting on the gland, it is obvious that much further work will be required in this field in order to be able to correlate changes in enzyme activity with secretory changes caused by nerves and hormones.

The submaxillary gland of the rat was found to belong to those salivary glands in which sympathetic stimulation produces a marked secretory response. The fact that the rapid flow of saliva elicited by maximal parasympathetic stimulation could not be further accelerated by excitation of the sympathetic fibres as well, was taken as evidence to show that those secretory cells which receive sympathetic secretory fibres have a parasympathetic innervation also. Histological investigations on the rat's submaxillary gland suggest that sympathetic fibres supply the acini and parasympathetic fibres both acini and tubules (Glimstedt and Hillarp, 1942; Snell and Garrett, 1958; Snell, 1960). In this connection it is interesting to find that procedures affecting the tubules may change the sensitivity of the gland to parasympathomimetic drugs. Hypo-

physectomy causes an atrophy of the tubules and was found to decrease the sensitivity to acetylcholine; treatment with thyroxine or testosterone is followed by hypertrophy of the tubules and increased acetylcholine sensitivity. After sympathetic denervation the acini are histologically changed, and the sensitivity is increased notably to sympathomimetics. Section of the parasympathetic fibres causes changes both in acini and tubules; supersensitivity develops both to parasympathomimetic and sympathomimetic agents.

This investigation was supported by grants from the Faculty of Medicine in Lund.

References

Ahlquist, R. P.: A study of the adrenotropic receptors. Amer. J. Physiol. *153:* 586–600 (1948).

Argonz, J. J.: The action of isoproterenol on the salivary glands. Acta physiol. latinoamer. *12:* 231–242 (1962).

Argonz, J. J. and Corral Saleta, J. M. de: Action de diverses hormones sur la glande sous-maxillaire du rat. C. R. Soc. Biol., Paris. *155:* 172 (1961).

Babkin, B. P.: Secretory mechanism of the digestive glands, 2nd ed. (1950). (Hoeber, New York).

Barka, T.: Induced cell proliferation: the effect of isoproterenol. Exp. Cell Res. *37:* 662–679 (1965).

Bernarde, M. A., Fabian, F. W., Rosen, S., Hoppert, C. A. and Hunt, H. R.: A method for the collection of large quantities of rat saliva. J. dent. Res. *35:* 326–327 (1956).

Bixler, D., Muhler, J. C., Webster, R. C. and Shafer, W. G.: Changes in submaxillary gland ribonucleic acid following hypophysectomy, thyroidectomy and various hormone treatments. Proc. Soc. exp. Biol., N. Y. *94:* 521–524 (1957).

Bixler, D., Webster, R. C. and Muhler, J. C.: The effect of pilocarpine on the submaxillary gland of the rat. J. dent. Res. *37:* 649–653 (1958).

Burgen, A. S. V. and Emmelin, N. G.: Physiology of the salivary glands. (1961). (Edward Arnold Ltd., London).

Chan, W. C.: Enlargement of the rat's salivary glands and salivary calculus formation induced with isoprenaline. J. Path. Bact. *88:* 563–574 (1964).

Drum, D. E.: Simple technique for direct cannulation of rat salivary ducts. J. dent. Res. *42:* 892 (1963).

Eckhard, C.: Beiträge zur Lehre von der Speichelsecretion. Z. rat. Med. *29:* 74–87 (1867).

Emmelin, N.: On spontaneous secretion of saliva. Acta physiol. scand. 30, Suppl. 111: 34–58 (1953).

– On the innervation of the submaxillary gland cells in cats. Acta physiol. scand. *34:* 11–21 (1955 a).

– Sympatholytic agents used to separate secretory and vascular effects of sympathetic stimulation in the submaxillary gland. Acta physiol. scand. *34:* 29–37 (1955 b).

– Action of transmitters on the responsiveness of effector cells. Experientia. *21:* 57–65 (1965).

Emmelin, N. and Engström, J.: Effect of sympathetic denervation on the sensitivity of the submaxillary gland to stimulating agents. J. Physiol. *153:* 9–16 (1960).

Emmelin, N., Holmberg, J. and Ohlin, P.: Receptors for catechol amines in the submaxillary glands of rats. Brit. J. Pharmacol. *25:* 134–138 (1965).

Emmelin, N., Jacobsohn, D. and Muren, A.: Effects of prolonged administration of atropine and pilocarpine on the submaxillary gland of the cat. Acta physiol. scand. *24:* 128–143 (1951).

Emmelin, N., Malm, L. and Strömblad, B. C. R.: Effect of denervation on the maximal secretory capacity of salivary glands. Quart. J. exp. Physiol. *45:* 349–351 (1960).

Emmelin, N. and Muren, A.: Sensitization of the submaxillary gland to chemical stimuli. Acta physiol. scand. *24:* 103–127 (1951).

– The sensitivity of submaxillary glands to chemical agents studied in cats under various conditions over long periods. Acta physiol. scand. *26:* 221–231 (1952).

Gabe, M.: Action de la thyroxine sur la glande sous-maxillaire du rat hypophysectomisé. C. R. Acad. Sci., Paris. *230:* 1317–1318 (1950).

Glimstedt, G. and Hillarp, N.-Å.: Über die Innervationsgebiete des Sympathikus und des Parasympathikus bei der Glandula submandibularis. Kungl. Fysiogr. Sällsk. Hand., N. F. *53:* 1–38 (1942).

Grad, B.: Joint actions of thyroxine and testosterone in thyroidectomized-castrated albino rats. Anat. Rec. *103:* 458 (1949).

Grad, B. and Leblond, C. P.: The necessity of testis and thyroid hormones for the maintenance of the serous tubules of the submaxillary gland in the male rat. Endocrinology. *45:* 250–266 (1949).

Hall, H. D. and Schneyer, C. A.: Salivary gland atrophy in rat induced by liquid diet. Proc. Soc. exp. Biol., N. Y. *117:* 789–793 (1964).

Handelman, C. S. and Wells, H.: Morphological and histochemical studies of experimentally enlarged and atrophied salivary glands of rats. Amer. J. Anat. *112:* 65–80 (1963).

Holloway, P. J. and Williams, R. A. D.: Investigation into oral secretions of rats after pilocarpine administration. J. dent. Res. *42:* 1087 (1963).

Houssay, A. B., Peronace, A. A. V., Perec, C. J. and Rubinstein, O.: Role of the chorda tympani in submaxillary hypertrophy by incisor amputation in the rat. Acta physiol. latinoamer. *12:* 153–166 (1962).

Jacobsohn, D., Larsson, S. and Norgren, A.: Enzyme activities in various organs of hypophysectomized rats and rabbits. Acta physiol. scand. *63:* 271–284 (1965).

Junqueira, L. C. U., Toledo, A. M. S. and Saad, A.: In Salivary Glands and their Secretions: 105–118 (1964). (Ed. L. M. Sreebny and Julia Meyer, Pergamon Press, New York).

Kahlson, G. and Renvall, S.: The distribution of cholinesterases in cats and changes caused by hypophysectomy, adrenalectomy, undernutrition and DOCA. Acta physiol. scand. *37:* 159–176 (1956).

Kronman, J. H.: A histochemical study of hypophysectomy-induced changes in rat submandibular and sublingual glands. Amer. J. Anat. *113:* 337–345 (1963).

Lacassagne, A.: Dimorphisme sexuel de la glande sous-maxillaire chez la souris. C. R. Soc. Biol., Paris. *133:* 180–181 (1940 a).

– Réactions de la glande sous-maxillaire à l'hormone male, chez la souris et le rat. C. R. Soc. Biol., Paris. *133:* 539–540 (1940 b).

Langenskiöld, A.: Component potentials of the submaxillary gland electrogram and their relation to innervation and secretion. Acta physiol. scand. *2*, Suppl. 6: 1–109 (1941).

Langley, J. N.: On the physiology of the salivary secretion. J. Physiol. *1:* 96–103 (1878–79).

Leblond, C. P. and Grad, B.: Control of the serous acini of the rat submaxillary gland by the thyroid hormone. Anat. Rec. *100:* 750 (1948).

Lundberg, A.: The electrophysiology of the submaxillary gland of the cat. Acta physiol. scand. *35:* 1–25 (1955).

Martin, K.: Observations on the increase in permeability induced by adrenaline in the submaxillary gland. J. Physiol. *172:* 50–60 (1964).

Norberg, K.-A. and Hamberger, B.: The sympathetic adrenergic neuron. Acta physiol. scand. *63*, Suppl. 238: 1–42 (1964).

Norberg, K.-A. and Olson, L.: Adrenergic innervation of the salivary glands in the rat. Z. Zellforsch. *68:* 183–189 (1965).

Nordenfelt, I. and Ohlin, P.: Supersensitivity of salivary glands of rabbits. Acta physiol. scand. *41:* 12–17 (1957).

Nordenfelt, I., Ohlin, P. and Strömblad, B. C. R.: Effect of denervation on respiratory enzymes in salivary glands. J. Physiol. *152:* 99–107 (1960).

Padykula, H.: The localization of succinic dehydrogenase in tissue sections of the rat. Amer. J. Anat. *91:* 107–145 (1952).

Potho, P. and Paasonen, M. K.: Studies on the salivary gland hypertrophy induced in rats by isoprenaline. Acta pharm. tox., Kbh. *21:* 45–50 (1964).

Schneider, R. M. and Person, P.: The isolation of submaxillary gland acini and duct segments. Exp. Cell Res. *20:* 627–629 (1960).

Schneyer, C. A.: Salivary gland changes after isoproterenol-induced enlargement. Amer. J. Physiol. *203:* 232–236 (1962).

Schneyer, C. A. and Schneyer, L. H.: Electrolyte levels of rat salivary secretions. Proc. Soc. exp. Biol., N. Y. *101:* 568–569 (1959).

– Secretion by salivary glands deficient in acini. Amer. J. Physiol. *201:* 939–942 (1961).

Selye, H., Veilleux, R. and Cantin, M.: Excessive stimulation of salivary gland growth by isoproterenol. Science. *133:* 44–45 (1961).

Shafer, W. G., Clark, P. G., Bixler, D. and Muhler, J. C.: Salivary gland function in the rat. I. Flow, viscosity, and pH in the normal and duct-ligated rat. J. dent. Res. *37:* 848–852 (1958).

Shafer, W. G., Clark, P. G. and Muhler, J. C.: The inhibition of hypophysectomy-induced changes in the rat submaxillary glands. Endocrinology. *59:* 516–521 (1956).

Shafer, W. G. and Muhler, J. C.: Effect of gonadectomy and sex hormones on the structure of the rat salivary glands. J. dent. Res. *32:* 262–268 (1953).

– Experimental dental caries. VI. The effect of hypophysectomy on dental caries and the salivary glands of the rat. J. dent. Res. *34:* 531–536 (1955).

– The effect of desiccated thyroid, propylthiouracil, testosterone, and fluorine on the submaxillary glands of the rat. J. dent. Res. *35:* 922–929 (1956).

– Endocrine influences upon the salivary glands. Ann. N. Y. Acad. Sci. *85:* 215–227 (1960).

Snell, R. S.: The effect of preganglionic parasympathectomy on the structure of the submandibular and major sublingual glands of the rat. Z. Zellforsch. *52:* 686–696 (1960).

Snell, R. S. and Garrett, J. R.: The effect of postganglionic sympathectomy on the structure of the submandibular and major sublingual salivary glands of the rat. Z. Zellforsch. *48:* 639–652 (1958).

Sreebny, L. M.: Histologic and enzymatic differences in the submaxillary glands of normal and hypophysectomized male and female white rats. J. dent. Res. *32:* 686 (1953).

Sreebny, L. M., Meyer, J., Bachem, E. and Weinmann, J. P.: Restoration of enzymatic activity in the submaxillary gland of the hypophysectomized albino rat. Endocrinology. *60:* 200–204 (1957).

Strömblad, B. C. R.: Acetylcholine splitting enzymes in salivary glands after prolonged treatment with pilocarpine and an atropine-like substance. Acta physiol. scand. *36:* 47–65 (1956 a).

– Amine oxidase in salivary glands after prolonged treatment with pilocarpine or an atropine-like substance. Acta physiol. scand. *36:* 158–170 (1956 b).

– Anaerobic glycolysis in normal and denervated salivary glands. Exp. Cell Res. *22:* 9–13 (1961).

Wells, H.: Submandibular salivary gland weight increase by administration of isoproterenol to rats. Amer. J. Physiol. *202:* 425–428 (1962).

Wells, H., Handelman, C. and Milgram, E.: Regulation by sympathetic nervous system of accelerated growth of salivary glands of rats. Amer. J. Physiol. *201:* 707–710 (1961).

Wells, H. and Peronace, A. A. V.: Synergistic autonomic nervous regulation of accelerated salivary gland growth in rats. Amer. J. Physiol. *207:* 313–318 (1964).

Wells, H. and Voelkel, E. F.: Submandibular salivary gland enlargement by feeding pancreatin to rats. Amer. J. Physiol. *205:* 1117–1121 (1963).

Wells, H., Zackin, S. J., Goldhaber, P. and Munson, P. L.: Increase in weight of the submandibular salivary glands of rats following periodic amputation of the erupted portion of the incisor teeth. Amer. J. Physiol. *196:* 827–830 (1959).

Pris kr. 5:—